

CANCER ANTINEOVASCULAR THERAPY

NAOTO OKU¹, TOMOHIRO ASAI¹, KOHTA KUROHANE¹, KOH WATANABE¹, KOICHI KUROMI¹, YUKIHIRO NAMBA², KOICHI OGINO² and TAKAO TAKI²

¹Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Yada, Shizuoka 422-8526 Japan; ²Nippon Fine Chemical Co. Ltd., Takasago, Hyogo 676-0074, Japan; and ³Molecular Medical Science Institute, Otsuka Pharmaceutical Co., Ltd., Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan.

Photodynamic therapy (PDT) is accomplished by laser light activation of accumulated photosensitizer in malignant tissues. This modality aims at selective damage to laser-irradiated tissues without damaging other parts of tissues. Benzoporphyrin derivative monoacid ring A (BPD-MA, verteporfin) is one of the second generation of photosensitizers for PDT. Since the concentration of BPD-MA in tumor tissue is higher than that in normal tissue at 3 h after the injection, PDT using BPD-MA is traditionally performed by laser irradiation at 3 h after intravenous injection of BPD-MA, and is effective against various tumor models. On the other hand, PDT using a proprietary formulation of BPD-MA is reported to affect microvasculature of tumor tissue as well as tumor cells, with blood flow stasis. Since angiogenesis is required for tumor growth in both primary and metastatic sites, PDT targeted to neovasculature may cause tumor regression through cutoff of nutrient and oxygen supply to tumor tissues due to hemostasis.

We investigated the effect of PDT with an experimental liposomal formulation of BPD-MA on tumor-induced angiogenic vessels using a murine dorsal air sac (DAS) model. At first, hemostasis of neovasculature was examined by varying the regimen of PDT. Laser irradiation at 15 min after injection of liposomal BPD-MA (15-min PDT) caused complete blocking of blood flow in neovasculature. In contrast, PDT did not inhibit blood flow when the irradiation occurred 3 h after the injection of liposomal BPD-MA (3-h PDT). The antitumor effect of PDT on Meth A sarcoma-bearing mice was far superior for the hemostasis-inducing regimen to that of conventional regimen, indicating that 15-min PDT causes strong suppression of tumor growth, possibly through damaging tumor neovascular endothelial cells rather than through a direct cytotoxic effect on tumor cells [1].

By the way, cytotoxic anticancer drugs cause damage to growing cells such as tumor cells. This characteristic often causes severe side effects through damaging normal growing cells such as marrow cells and intestinal cells. Since angiogenic endothelial cells have growing character, these cells may also be damaged by cytotoxic anticancer drugs. Anti-neovascular therapy (ANT) may cause indirect lethal damage of tumor cells through the damage of newly formed blood vessels with reduced side effects, like as the case of PDT shown above.

Moreover, tumor cells often acquire drug-resistance, however, neovascular endothelial cells would not be expected to acquire drug-resistance. Thus we tried to develop liposomal DDS drugs which target angiogenic vessels, and applied the system for ANT.

At first, we isolated peptides specific for tumor angiogenic vasculature using a phage-displayed peptide library, to develop a targeting probe for neovasculature. We injected a phage-displayed peptide library into angiogenesis model mice prepared by DAS method instead of tumor-bearing mice, and isolated phage clones specifically accumulated in angiogenic site. The advantage of this *in vivo* biopanning method using DAS model mice is that the selected phages have the ability to bind only to angiogenic vessels, not to tumor cells. In fact, the amino acid sequences of the phage clones thus obtained were different from any reported sequences obtained by *in vivo* biopanning with tumor-bearing mice. The selected phage clones had high affinity to murine angiogenic vessels. By the method, we isolated three distinct phage clones that markedly accumulated in murine tumor. After the determination of the epitope sequences of these peptides, we modified liposomes with epitope penta-peptides. Liposome modified with APRPG-peptide showed high accumulation in murine tumor, and APRPG-modified liposome encapsulating adriamycin effectively suppressed experimental tumor growth [2], suggesting ANT is useful for cancer treatment. Since the therapeutic efficacy by ANT using adriamycin-encapsulated liposomes reflects the damage of the cells through the change in local concentration of the agent in tumor tissue, ANT by using lipophilic anti-cancer drug was nextly examined. In this case, the lipophilic drugs should be delivered to the cells as liposomal form. The therapeutic efficacy of APRPG-liposomal anticancer drug, dipalmitoylphosphatidyl-CNDAC, was examined. This liposomal drug effectively suppressed tumor growth compared to non-modified liposomal DPP-CNDAC, suggesting that the destruction of angiogenic endothelial cells is superior to the direct destruction of tumor cells in the tumor treatment. Furthermore, we investigated the applicability of APRPG-liposomes to human. The specific binding of APRPG-modified liposome to human umbilical endothelial cells, and that of PRP-containing peptide to angiogenic vessels in human tumors, i.e., islet cell tumor and glioblastoma, were clearly observed. The present study provides a novel modality of cancer treatment, ANT, and the usefulness of APRPG-modified liposomes as a drug carrier for ANT.

REFERENCES

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